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Computerized Preference-Avoidance Quantification and Analysis: A Rapid, High-Resolution Method

E. Scherer, R.V. Hunt and R.E. McNicol

Central and Arctic Region
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by

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ABSTRACT

SCHERER, E., R.V. HUNT, and R.E. McNICOL. 1989. Computerized preference-avoidance quantification and analysis: a rapid, high-resolution method. Can. Tech. Rep. Fish. Aquat. Sci. 1672: iv + 9 p.

Preference-avoidance responses are widely used in sublethal toxicity assessments. Response quantification and analysis are as yet non-standardized and problematic. A previously published tracking device delivers analog recordings of animal movements between "pure" and chemically or physically altered test areas. For lack of cost- and labour-efficient methods, it has not been feasible so far to adequately utilize the information contained in such recordings. This report describes an inexpensive add-on module consisting of an analog-digital converter, microcomputer, and printer. The addition provides instantaneous, automatic and complete quantification of animal movements in choice experiments. The customary percent-time criterion can now be easily supplemented or supplanted by information on constituent and underlying parameters, such as movement frequencies and velocities into and away from different test areas, chemokinetic effects, and distances travelled. Possible artifacts such as wall effects can be identified and quantified. Efficiency, accuracy and precision of the method suggest its usefulness for both research and routine testing applications.

Key words: behavior: locomotion; fish: toxicant; sublethal response; digitization.

RÉSUMÉ

SCHERER, E., R.V. HUNT, and R.E. McNICOL. 1989. Computerized preference-avoidance quantification and analysis: a rapid, high-resolution method. Can. Tech. Rep. Fish. Aquat. Sci. 1672: iv + 9 p.

Les réactions de préférence-évitement sont largement utilisées dans les évaluations de la toxicité sublétale. Cependant, la quantification et l'analyse des réponses n'est pas encore normalisée et reste une source de difficultés. Un dispositif conçu pour suivre les déplacements d'un animal et ayant fait l'objet d'une publication antérieure fournit un enregistrement analogique des mouvements de l'animal entre des zones d'essai "pures" et des zones d'essai où les conditions chimiques ou physiques ont été modifiées. En l'absence de méthodes économiques et peu fastidieuses, il n'a pas été possible jusqu'ici d'exploiter pleinement l'information contenue dans ces enregistrements. Le présent rapport décrit un module peu coûteux que l'on peut ajouter au dispositif de localisation et qui comprend un convertisseur analogique-numérique, un microordinateur et une imprimante. L'addition de ce module permet une quantification instantanée, automatique et complète des mouvements de l'animal au cours des expériences faisant intervenir un choix de l'animal. Le critère traditionnel du pourcentage de temps peut maintenant être facilement complété ou supplanté par de l'information sur les paramètres constituants et sous-jacents, tels les fréquences et les vitesses de déplacement entre les différentes zones d'essai, les effets chimiocinétiques et les distances parcourues. Les artéfacts possibles comme les effets de mur peuvent être reconnus et quantifiés. À cause de son efficacité, de sa précision et de sa fidélité, la méthode sera utile aussi bien pour la recherche que pour l'analyse usuelle.

Mots-clés: comportement; locomotion; poisson; toxiques; réponse sublétale; numérisation.

INTRODUCTION

Preference-avoidance testing has become one of the more frequently used means to assess sublethal effects of environmental alterations on aquatic organisms (see reviews by Cherry and Cairns 1982; Giattina and Garton 1983; Beitinger and Freeman 1983; Rand 1985). As with many behavioral tests, accurate and efficient quantification and analysis of responses have been challenging problems (Lawrence and Scherer 1974; Scherer 1977; de March and Scherer 1980; Scherer, in press). The most easily and frequently measured parameter is the proportion of time spent by the test organisms in "pure" (unaltered) versus altered (contaminated) areas. This integrative parameter does not reveal information on underlying or associated responses; considered in isolation, it can be quite misleading. For instance, a slowing-down of the test organism caused by a toxic compound (a chemokinetic effect) can be misinterpreted as "preference" (Sprague and Drury 1969; Lawrence and Scherer 1974; de March and Scherer 1980). Therefore, most authors use direct observations or examine recordings to interpret more properly any "time-spent" effect.

To obtain accurate recordings, we previously developed an easy-to-use, efficient tracking device (Scherer and Nowak 1973) that has since been employed by several workers (e.g., Lawrence and Scherer 1974; Scherer 1975; Hara and Thompson 1978; Tatsukawa and Hidaka 1978; Hodgins and Maciorowski 1979; Kamchen and Hara 1980; Jones et al. 1985; Hidaka and Tatsukawa 1985). However, until now the information contained in such recordings has never been adequately utilized. It was possible, but prohibitively slow and labour-intensive to compute from strip chart recordings even basic locomotor response elements such as swim velocities and their changes. Recent technical developments made it feasible to devise a simple and inexpensive computerized addition to the existing tracking apparatus, thereby turning it into a full-fledged data acquisition system that is directly interfaced with a microprocessor. This new system will be described in the following.

THE APPARATUS

Figure 1 illustrates the counter-current type avoidance trough as used in our laboratory in conjunction with the previously published tracking device (Scherer and Nowak 1973).

The trough design is similar to those employed by Sprague (1964) and Jones (1947), which in turn go back to Shelford and Allee (1913). Water is pumped into a glass or clear acrylic tank from both ends at equal rates, draining at the center. With velocities set at < 1 cm·s^{-1}, separation of the two water bodies at the tank centre is achieved without impeding the movements of test species (fish or macroinvertebrates, e.g. Maciorowski et al. 1977).

The tracking device employs a simple gunsight type viewer which is connected to a power-supplied potentiometer in such a way that movements of the viewer by the experimenter produce varying voltage outputs (Fig. 1B). Thus, a certain position of the test animal within the test area corresponds to a certain voltage value, the basic parameter for response quantification.

Figure 2 illustrates connections between elements of the previous (Scherer and Nowak 1973) and the present, extended apparatus. In the original system, a strip chart recorder was the only output-gathering device. It produced analog records of the test animal's movements, similar (if less precise and accurate) to that reproduced in Fig. 5 which is plotted by computer from automatically digitized position voltages sampled at ≈ 1 s intervals.

The digitizer is an Omega Inc. WB-31 "White Box"® analog-to-serial interface. Similar models by other manufacturers may perform similarly well. However, no comparative tests have been carried out. The WB-31 accepts analog inputs with a standard range (used here) of ± 5 Vdc (alternatively 20 V max) and a standard resolution of 1 part in 5000 equalling 1 mV (alternatively 1 part in 20000 or 0.25 mV max). Transmission rates between 110 and 9600 baud are possible. The analog to digital conversion rate is 7.5 per second, equivalent to 133 ms per conversion. Only a simple BASIC program (e.g. Fig. 3) is needed to store data generated by the WB-31, which works as a computer peripheral. Figure 4 shows a printout of fish positions in the form of digitized voltages as produced by the Omega "White Box"®.

For data collection and storage, the simplest and least expensive microcomputer system available may be used. Most computers on the market today have a RS-232 port which connects with the "White Box"® digitizer. We used a Commodore 64C with a 1541-II disk drive and monochrome display. At 64 K of RAM, the 64C is at the lower end of modern-day microcomputers (both in memory capacity and price), yet it proved entirely adequate for the present purpose. A buffer capacity of ≈ 1100 entries limits the length of a continuous recording period (see Discussion) because there is a ≈ 15 s interruption while data are being loaded onto diskette. The 5.25" diskettes formatted by the 1541-II drive module have a total capacity of 174848 bytes each; 65535 records per file, 144 files per diskette. This means ample capacity for storing programmes and results, e.g. the program reproduced in Fig. 3 requires 7 blocks; each 15 min avoidance test file (920 records) occupies Σ 40 blocks. With 666 blocks available on a formatted diskette, about 14 avoidance test files including programs can fit on one diskette.

DISCUSSION

The system described here may be used to analyze not only preference-avoidance responses, but any linear or one-dimensionally projected

movements. Two- or three-dimensional analyses would require more elaborate systems, possibly employing viewers with potentiometers mounted in two or three planes, and/or using mirrors that transpose planes of view.

After technical checks and "dry-runs", we applied the present system to a set of experiments testing the preference-avoidance response of lake whitefish, Coregonus clupeaformis, to sublethal concentrations of cadmium. The system performed flawlessly. We chose a duration of 15 min per test and control replicate, with a position sampling frequency of $\approx 1 \cdot s^{-1}$. This procedure yielded 920 data entries per replicate, safely within the 64C's buffer limit and in line with durations most authors apply. Accuracy and precision exceeded normal requirements; position differences in the millimeter range were recorded with practically absolute precision. There was no drift that usually occurs with strip chart recorders. As previously suggested (Scherer and Nowak 1973; Lawrence and Scherer 1974; de March and Scherer 1980) we quantified and analyzed several response parameters beyond the customary "time-spent" criterion, e.g. swim velocities into and out of the two tank sides, "trip times" (time from entering to leaving one side and the other), depths of penetration (see Fig. 6; McNicol and Scherer, unpubl.). This additional information identifies the various behavioral responses that lead to differences in lengths of time spent in one tank half and the other. Moreover, it prevents misinterpretations, e.g. an apparent "preference" as indicated by the time-spent criterion above may actually be based on strong locomotor inhibition by a so-called wall effect, or on stupefaction caused by the test toxicant (e.g. Jones 1947; Sprague and Drury 1969). Such particular responses (also changes of swim velocities represented by slopes) can be recognized by visually screening tracings as reproduced in Fig. 5. If there is access to mainframe computers, data can be readily transferred from microcomputer diskettes to the mainframe with its extended capacity for any kind of elaborate statistical processing and graphics output. Instant and automatic digitization (Fig. 4) and subsequent processing using commercially available statistical software (e.g. SAS , SAS Institute Inc., Cary, NC.) make our method rapid, accurate, and precise.

LIST OF PARTS AND PRICES

Approximate prices are in 1989 Canadian dollars.

	$
1. Dosing pump, tanks, plumbing . . .	800
2. Electronics for tracking module . .	150
3. A/D converter (here: Omega WB-31) .	600
4. Microcomputer (here: Commodore 64C).	200
5. Disk drive (here: Commodore 1541-II)	380
6. Printer	500

A package with details for construction of the test tank and assemblage of items 1 and 2 is available on request.

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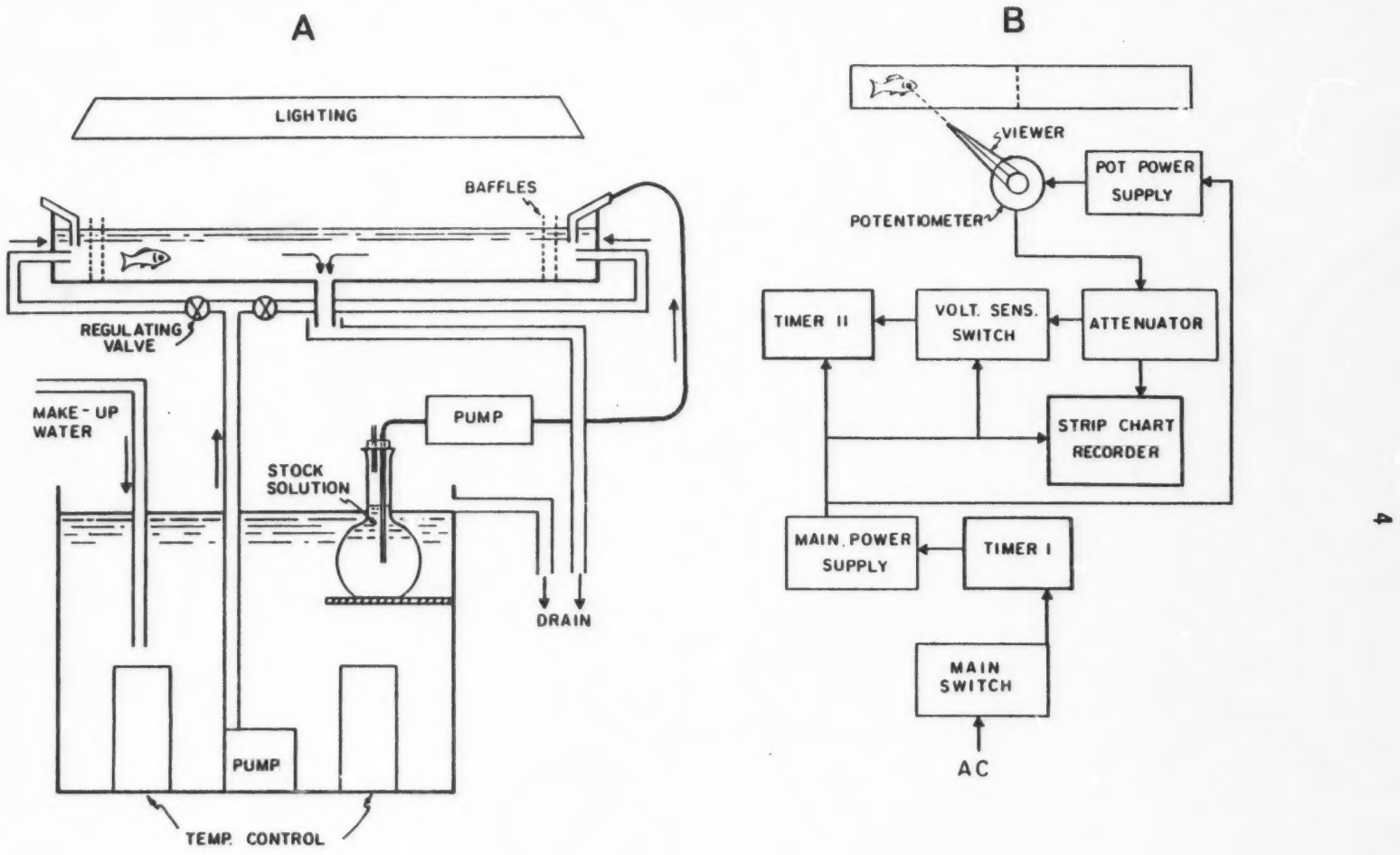

Fig. 1 A,B. Schematic of tank (A) and block diagram of recording assembly (B). From Scherer and Nowak (1973).

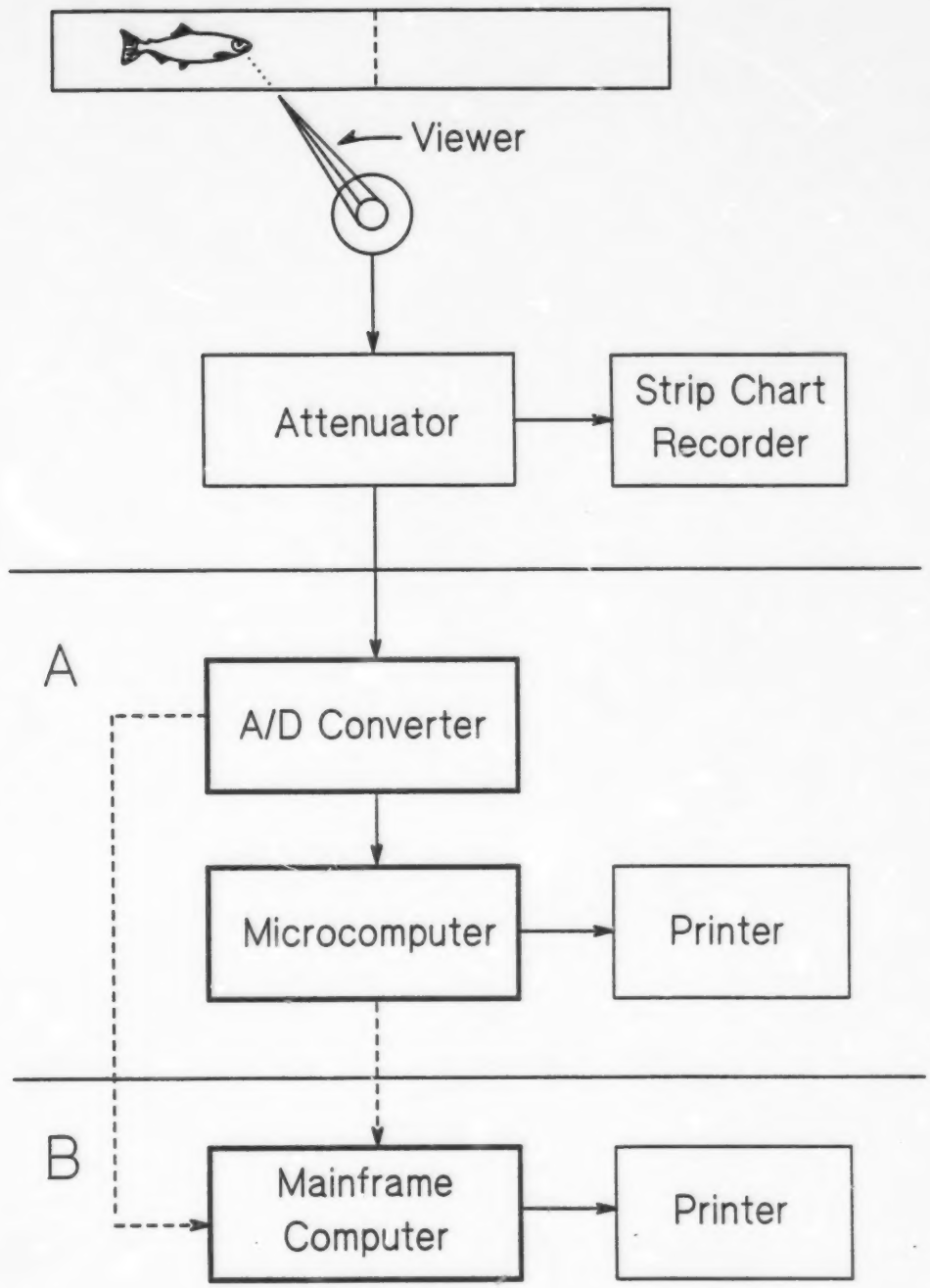

Fig. 2 A,B. Block diagram of new computerized system.
A : basic option using microcomputer.
B : option using connection to mainframe.

```
5 REM WHITEBOX DATA CAPTURE PROGRAM
10 REM SAVES POSITION DATA TO DISK AFTER 920 READINGS
15 OPEN 2,2,3,CHR$(38)+CHR$(64)   :REM DIRECTS PORT INPUT TO RS232 ROUTINE
18 DIM D(2000), TM(2000):CT=1:N1=920
80 PRINT "□ENTER NUMBER OF WHITEBOX CONVERSIONS BETWEEN REQUIRED"
81 PRINT "                                              POSITION MEASUREMENTS"
85 INPUT"3■■■■";S
100 PRINT"□PRESS ■F1■ WHEN READY TO LOG DATA"
101 PRINT
102 PRINT"      ■F7■ TO STOP LOGGING"
104 PRINT
110 GET K$:IF K$=CHR$(133) THEN GOTO 125      :REM WAITS FOR AN F1 KEY
120 GOTO 110
125 PRINT "COUNTER","TIME","MILLIVOLTS"
130 T0=TI/60:N=1
140 GET#2,C$                          :REM OBTAINS A CHARACTER FROM RS232 BUFFER
142 GET KY$:IF KY$=CHR$(136) THEN GOSUB 5000:CLOSE2:END
155 IF C$=CHR$(13)THEN GOTO 190   :REM BUILDS A STRING OF CHARACTERS UNTIL
160 T$=T$+C$                          :REM A CARRIAGE RETURN IS RECEIVED
170 GOTO 140
190 IF N<S GOTO 275
235 D(CT)=VAL(RIGHT$(T$,6))       :REM CONVERTS EVERY S-TH CONVERSION STRING TO
236 TM(CT)=INT(TI/60-T0)          :REM NUMERIC ARRAY FORMAT
244 PRINT CT,TM(CT),D(CT)
245 N=0
247 F=FRE(0)
250 GET K$:IF K$=CHR$(136) THEN GOSUB 5000:CLOSE2:END:REM EXITS PROGRAM ON F7
260 IF CT=N1 THEN GOSUB 5000:CLOSE 2:END
270 CT=CT+1
275 T$="":N=N+1                       :REM DISCARD CURRENT CONVERSION STRING
300 GOTO 140                          :REM INCREMENT CONVERSION COUNTER
5000 REM PERMANENT FILE STORAGE**************************************************
5010 OPEN 15,8,15
5020 PRINT#15,"S0:DATA"
5030 OPEN 6,8,6,"0:DATA,S,W"
5040 FOR I=1 TO CT
5050 PRINT#6,STR$(TM(I))+" "+STR$(D(I));CHR$(13);
5060 NEXT I
5070 CLOSE 6:CLOSE 15
5080 PRINT "□PERMANENT FILE STORAGE Y/N?"
5090 INPUT Y$
5100 IF Y$="N" THEN RETURN
5110 IF Y$="Y" THEN GOTO 5130
5120 GOTO 5080
5130 INPUT "FILE NAME?";FI$
5140 OPEN 15,8,15
5150 PRINT#15,"C0:"+FI$+"=DATA"
5160 CLOSE 15
5170 RETURN
6000 REM TO READ DATA FILE TYPE RUN 6000 ************************************
6010 OPEN 5,8,5,"0:DATA,S,R"
6020 INPUT#5,B$
6030 PRINT B$
6040 IF ST=0 THEN GOTO 6020
6050 CLOSE 5:CLOSE15:END

READY.
```

Fig. 3. Example: a simple BASIC step program for running a preference-avoidance test.

①		②		③		④	
200	.76	646	.484	308	.796	665	.811
201	.802	647	.402	309	.827	666	.815
202	.811	648	.352	310	.828	667	.809
203	.815	649	.316	311	.818	668	.826
204	.81	650	.272	312	.819	669	.83
205	.81	651	.197	313	.821	670	.836
206	.813	652	.214	314	.802	671	.845
207	.816	653	.227	315	.793	672	.842
208	.816	654	.212	316	.801	673	.841
209	.809	655	.207	317	.823	674	.83
210	.812	656	.223	318	.819	675	.824
211	.811	657	.311	319	.807	676	.827
212	.801	658	.372	320	.804	677	.825
213	.806	659	.421	321	.799	678	.824
214	.801	660	.531	322	.802	679	.82
215	.808	661	.614	323	.799	680	.816
216	.805	662	.654	324	.803	681	.812
217	.803	663	.721	325	.803	682	.811
218	.798	664	.736	326	.798	683	.805
219	.799	665	.743	327	.803	684	.801
220	.8	666	.755	328	.801	685	.8
221	.798	667	.76	329	.794	686	.802
222	.803	668	.79	330	.677	687	.799
223	.801	669	.795	331	.634	688	.83
224	.793	670	.759	332	.576	689	.801
225	.793	671	.744	333	.495	690	.787
226	.749	672	.728	334	.433	691	.803
227	.745	673	.64	335	.359	692	.799
228	.67	674	.555	336	.314	693	.807
229	.647	675	.495	337	.316	694	.832
230	.62	676	.441	338	.3	695	.725
231	.619	677	.376	339	.265	696	.66
232	.649	678	.35	340	.237	697	.581
233	.685	679	.342	341	.222	698	.547
234	.715	680	.304	342	.228	699	.545
235	.731	681	.291	343	.226	700	.514
236	.759	682	.29	344	.228	701	.465
237	.782	683	.233	345	.311	702	.389
238	.781	684	.206	346	.323	703	.337
239	.807	685	.22	347	.328	704	.313
240	.802	686	.223	348	.381	705	.278
241	.806	687	.226	349	.441	706	.263
242	.804	688	.314	350	.508	707	.245
243	.801	689	.505	351	.541	708	.241
244	.806	690	.594	352	.617	709	.23
245	.798	691	.655	353	.658	710	.223
246	.794	692	.719	354	.712	711	.307
247	.781	693	.767	355	.759	712	.385
248	.788	694	.783	356	.812	713	.426
249	.798	695	.791	357	.838	714	.511
250	.794	696	.813	358	.855	715	.544
251	.805	697	.793	359	.865	716	.59
252	.803	698	.799	360	.853	717	.66
253	.803	699	.783	361	.853	718	.741
254	.805	700	.772	362	.853	719	.76
255	.8	701	.686	363	.852	720	.791
256	.804	702	.526	364	.858	721	.799
257	.742	703	.404	365	.853	722	.8
258	.647	704	.3	366	.86	723	.843
259	.558	705	.246	367	.86	724	.793
260	.503	706	.234	368	.863	725	.795

Fig. 4. Printouts of digitized fish positions; left-hand columns: running time records; right-hand columns: voltages representing fish positions. Columns 1 to 4 correspond to sections 1 to 4 (between arrows) in Fig. 5A,B.

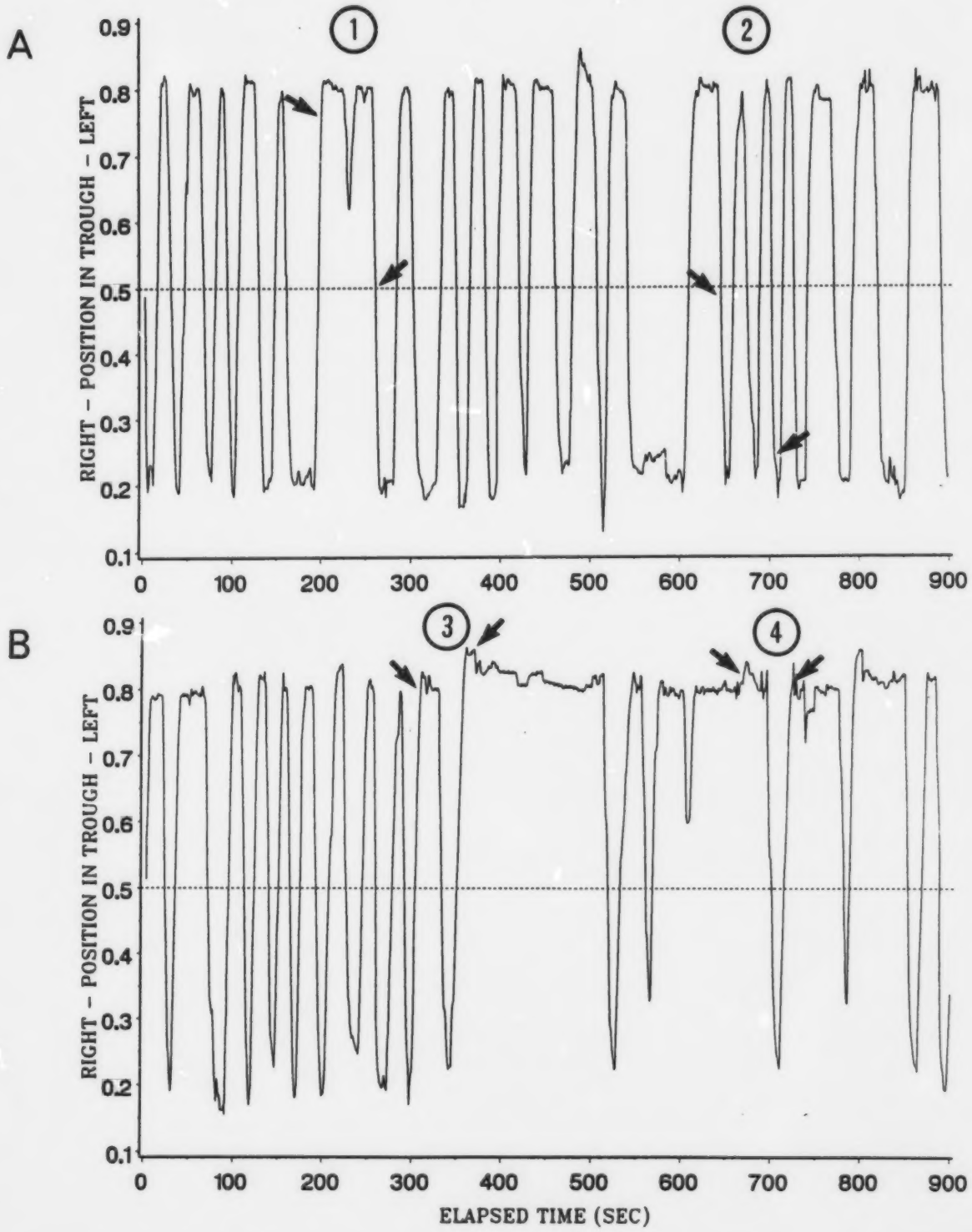

Fig. 5 A,B. Tracings of lake whitefish (Coregonus clupeaformis) positions, produced by a SAS/GRAPH® program using digitized data partially printed out in Fig. 4. A: control run, pure water in both tank halves; B: test run, 0.5 $\mu g \cdot L^{-1}$ cadmium in right half of tank.

LEFT= 23	DEPTH= 290	VELOCITY OUT, LEFT= 88	RATIO OUT:IN,LEFT= 1.875
RIGHT= 11	DEPTH= 290	VELOCITY OUT, RIGHT= 61	RATIO OUT:IN,RIGHT=0.833
LEFT= 38	DEPTH= 290	VELOCITY OUT, LEFT= 40	RATIO OUT:IN,LEFT= 5.333
RIGHT= 25	DEPTH= 290	VELOCITY OUT, RIGHT=122	RATIO OUT:IN,RIGHT=1.778
LEFT= 17	DEPTH= 290	VELOCITY OUT, LEFT= 84	RATIO OUT:IN,LEFT= 3.250
RIGHT= 11	DEPTH= 290	VELOCITY OUT, RIGHT= 60	RATIO OUT:IN,RIGHT=1.200
LEFT= 17	DEPTH= 290	VELOCITY OUT, LEFT= 99	RATIO OUT:IN,LEFT= 2.400
RIGHT= 11	DEPTH= 275	VELOCITY OUT, RIGHT= 54	RATIO OUT:IN,RIGHT=0.571
LEFT= 13	DEPTH= 290	VELOCITY OUT, LEFT= 72	RATIO OUT:IN,LEFT= 1.600
RIGHT= 12	DEPTH= 290	VELOCITY OUT, RIGHT=108	RATIO OUT:IN,RIGHT=1.400
LEFT= 18	DEPTH= 290	VELOCITY OUT, LEFT= 60	RATIO OUT:IN,LEFT= 2.000
RIGHT= 15	DEPTH= 290	VELOCITY OUT, RIGHT= 59	RATIO OUT:IN,RIGHT=1.500
LEFT= 20	DEPTH= 290	VELOCITY OUT, LEFT= 43	RATIO OUT:IN,LEFT= 2.333
RIGHT= 18	DEPTH= 254	VELOCITY OUT, RIGHT= 45	RATIO OUT:IN,RIGHT=0.286
LEFT= 15	DEPTH= 290	VELOCITY OUT, LEFT= 186	RATIO OUT:IN,LEFT= 2.000
RIGHT= 19	DEPTH= 290	VELOCITY OUT, RIGHT= 70	RATIO OUT:IN,RIGHT=2.167
LEFT= 14	DEPTH= 290	VELOCITY OUT, LEFT= 102	RATIO OUT:IN,LEFT= 0.750
RIGHT= 13	DEPTH= 290	VELOCITY OUT, RIGHT= 52	RATIO OUT:IN,RIGHT=1.167
LEFT= 29	DEPTH= 290	VELOCITY OUT, LEFT= 81	RATIO OUT:IN,LEFT= 4.800
RIGHT= 18	DEPTH= 278	VELOCITY OUT, RIGHT= 67	RATIO OUT:IN,RIGHT=1.000
LEFT= 172	DEPTH= 290	VELOCITY OUT, LEFT= 76	RATIO OUT:IN,LEFT= 23.57
RIGHT= 14	DEPTH= 278	VELOCITY OUT, RIGHT= 72	RATIO OUT:IN,RIGHT=0.556
LEFT= 30	DEPTH= 290	VELOCITY OUT, LEFT= 87	RATIO OUT:IN,LEFT= 1.500
RIGHT= 7	DEPTH= 173	VELOCITY OUT, RIGHT= 87	RATIO OUT:IN,RIGHT=0.400
LEFT= 136	DEPTH= 290	VELOCITY OUT, LEFT= 49	RATIO OUT:IN,LEFT= 18.43
RIGHT= 13	DEPTH= 277	VELOCITY OUT, RIGHT= 85	RATIO OUT:IN,RIGHT=0.300
LEFT= 69	DEPTH= 290	VELOCITY OUT, LEFT= 108	RATIO OUT:IN,LEFT= 7.625
RIGHT= 6	DEPTH= 177	VELOCITY OUT, RIGHT= 57	RATIO OUT:IN,RIGHT=0.200
LEFT= 67	DEPTH= 290	VELOCITY OUT, LEFT= 64	RATIO OUT:IN,LEFT= 15.75
RIGHT= 16	DEPTH= 281	VELOCITY OUT, RIGHT= 68	RATIO OUT:IN,RIGHT=0.333
LEFT= 20	DEPTH= 290	VELOCITY OUT, LEFT= 136	RATIO OUT:IN,LEFT= 4.000

TOTAL TIME ON LEFT SIDE	698
NO. OF LEFT SIDE TRIPS	16
PERCENT TIME ON LEFT	75.86957
MEAN LEFT-SIDE TRIP TIME	43.625
MEAN DEPTH OF PENETRATION,LEFT	290
MEAN VELOCITY OUT LEFT/IN RIGHT	85.9375
MEAN RATIO OUT:IN,LEFT	6.076042
MEAN TOT RATIO OUT:IN,LEFT	5.98

TOTAL TIME ON RIGHT SIDE	222
NO. OF RIGHT SIDE TRIPS	15
PERCENT TIME ON RIGHT	24.13043
MEAN RIGHT-SIDE TRIP TIME	13.93333
MEAN DEPTH OF PENETRATION,RIGHT	268.2
MEAN VELOCITY OUT RIGHT/IN LEFT	71.13333
MEAN RATIO OUT:IN,RIGHT	0.9126984
MEAN TOT RATIO OUT:IN,RIGHT	0.8333333

Fig. 6. Printout of response data, derived from digitized fish positions of tracing shown in Fig. 5B.